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Original Article

The Biological Safety of Stainless Steel Needles Used in Warm-needling

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Warm-needling (also called thermo-acupuncture) is a combination of acupuncture and moxibustion. Due to the intense heat involved, there have been concerns over the biological safety of the acuneedles used in the treatment. This paper reports two phases of a safety test. For a preliminary test, we compared the temperature change patterns of stainless steel (SS304) needles and traditional gold alloy needles, which have been increasingly replaced by the former. To verify the effects of the presence of coating materials, the main test involved three different kinds of SS304: silicone-coated, salicylic acid-coated and non-coated needles. Each group of needles was tested for pH level, heavy metals and UV absorbance spectrum along with biological tests on the cytotoxicity and hemolysis of the needle. All the tests on the extractants from the needles were negative. In the biological tests, each test result showed a significant difference from the positive control samples, while no significant difference was observed compared with the negative control samples. In the hemolysis tests, all samples satisfied the Korean Government Standards. All the results suggest that SS304 needles are biologically safe to be used in warm-needling, though they can be improved to perform as well as the gold alloy needles in terms of temperature fluctuations.

Keywords: acupuncture – moxibustion – SS304 needle – warm needle – warm-needling

Introduction

Warm-needling (also called thermo-acupuncture) is one way to administer indirect moxibustion. A needle is inserted into an acupoint and on top of the needle moxa is attached and burned to provide heat via the needle. Warm-needling has been used as a useful therapeutic measure to improve chronic diseases generating from irregular Qi flows caused by a lack of heat. Traditionally, a special kind of gold alloy needle has been custom-made for the treatment. However, because of their high availability and low price, ordinary stainless steel (SS304) needles have increasingly replaced the gold alloy needles in clinical treatments.

It has been well established that SS304 needles are biologically safe and harmless to humans (1–3). However, a case of silicone granuloma arising on the entry points of acupuncture has been reported (4). The needles used in warm-needling are exposed to intense heat in the procedure and thus they are suspected to undergo some transformation in their chemical and mechanical composition. Since SS304 are composed of various metal elements like C, Si, P, S, Cr, Mn, Fe and Ni (5), there is a concern that the heat might help release the metal elements into the patient's body and cause biologically

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hazardous conditions. We tested whether SS304 needles, when exposed to intense heat, are biologically safe and harmless to humans.

This study was designed to verify the biological safety of the SS304 needles used in warm-needling. As a preliminary test, the patterns of temperature change in the needles of different materials were observed. Then biological tests and hemolyses were conducted with extractants from the needles. The test protocol was based on the common standard of Korean Food and Drug Administration (KFDA) (6).

Materials and Methods

Materials

To verify the temperature change patterns of acuneedles made of different materials two kinds of needles were used: ordinary silicone-coated SS304 needles [Tongbang Acuneedle and Moxa Manufacturer (TAMM), Korea 0.25×40 mm, 0.1478 g] and special gold acuneedles used exclusively for warm-needling (TAMM, Korea 0.53×35 mm, 95% gold and 5% white gold needle with 1.14×33 mm 100% silver moxa chamber attached; total weight, 0.4765 g). The heat was provided by burning moxa (Inwhadang, Korea) (Fig. 1). The moxa was measured with a CP2 24S scale (SARTORIUS AG, Germany) and shaped into a 0.50 ± 0.01 g ball with a 20 mm diameter.

Three kinds of SS304 needles were selected in the biological safety test: the silicone-coated, the noncoated (the same needles, but the coating was removed with 70% ethyl alcohol solution) and the salicylic-acid-coated (Korean Traditional Medicine Project Corps, Korea, 0.25×40 mm).

Figure 1. The photo of the warm-needling.

Methods

Temperature Change Patterns in Different Kinds of Needles

The three needles were fixed vertically with moxa burning from the top of the moxa chamber. By using a thermo couple, the temperatures in two different points on the acuneedle (10 mm and 20 mm from the bottom of the moxa chamber) were measured. The room temperature was 24 ± 2°C.

pH Values

Silicone-coated needles, salicine acid-coated needles and coating-removed needles were put in a bottle containing 300 ml of distilled water with a proportion of 15 g needles to 100 ml solution. Three hundred needles, 100 of each kind, were used. The bottle was then sealed with para film. The extraction process was conducted at three different temperatures: 37°C, 50°C and 70°C. The duration of each process was 60 ± 2 min. In a bottle containing 20 ml of distilled water and 20 ml of the extractants, 1.0 ml of 1 g/l NaCl solution was added and then the pH values were measured three times with a pH meter (Model 420, Thermo Orion, USA). To meet the KFDA standards, the pH value had to be below pH 1.0.

Heavy Metal Test

The extractant solutions were prepared in the same manner as in two, except for the durations: 72 h at 37°C, 48 h at 50°C and 24 h at 70°C. With the extractant solutions from the silicone and the salicine-coated needles, colormetric comparison tests were carried out. 20 ml of the extractant solution or distilled water was put in a 50 ml tube. Then, one drop of phenolphtalane solution (100 ml ethanol + 1 g phenolpetalane) and 2 ml of acetic acid solution (100 ml distilled water + 36 g acetic acid) were added. To the standard solution, 20 ml of 10 p.p.m. lead solution was added. Tubes containing extractant solution or standard solution received 0.1 N nitric acid to reach 50 ml 5 min after one to two drops of sodium sulfide solution (5 g sodium sulfide + 10 ml water + 30 ml glicerine) were added to all the tubes; then the colormetric comparison was conducted.

With the extractant solutions from the silicone and the salicine-coated needles, Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES) was conducted. As the KFDA standards recommend, Cu, Fe, Pb and Sn were measured. The wavelength ranges were 327.395, 238.204, 220.353 and 283.99 nm respectively. The analyzer was Vista-Pro (Varian, Australia; 1200W output, 15.0 l/min plasma flow, 1.5 l/min auxiliary flow, 0.7 l/min nebulizer flow).

Ultraviolet Absorbance Spectrum Measurement

With the same extractant solutions, the absorbance measurements were taken at 250 nm wavelength. SCINCO 3100 (SCINCO, Korea) was used in the analysis. The concentration of the test solution was 100 p.p.m. Background correction was set with the built-in system of the machine. To remove noise, the background was measured before each test and subtracted from the raw data after the test. The raw data were collected 25 times.

Biological Test: Tetrazolium-based Colormetric Test

The Tetrazolium-based Colormetric Test (MTT) was carried out to test the cytotoxicity of the needles. The same three kinds of needles were tested with Cu as the positive control and SS304 as negative control. The extractant solutions were prepared in the same manner as in two, except for Cu solution, which was prepared for 24 h at 70°C in 0.9% NaCl solution.

For the test CCL81 (Vero) cells were obtained from Seoul National University Cell Lines Bank, Korea. CCL81 cells were cultivated with RPM1640 Media containing 10% fetal bovine serum in a 5% CO2 cultivator at 37°C. Passaging was carried out once every 2–3 days. Cells were seeded in a 96-well plate and cultured for 24 h. After the initial 24 h, the extractant solutions were put into the wells and the cells were cultured for another 48 h.

After 48 h of culturing, 10 μl 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) and 90 μl Media were put into the wells. After another 2 h of culturing in the incubator at 37°C, 100 μl DMOS was added. Cell viability was measured with ELISA reader at 490 nm after dissolving formazan.

Biological Test: Hemolysis Test

Distilled water was used as the positive control, and saline water was used as the negative control. The extractant solution was obtained in the same manner as in the blood sample from a male rabbit were collected in vacuum blood collection tubes containing EDTA within 1 h. The 15 ml blood samples were diluted with 20 ml 0.9% NaCl solution. Five milligram of each extractant solution was added to a 10 ml 0.9% NaCl solution inside a vial. The negative control and the positive control were prepared in different vials with 0.2 ml of the diluted blood sample in each of them. All the vials were left in 37°C water for 60 min, and then centrifuged for 5 min at 500×. The solutions at the top of the vial were tested for absorbance at 540 nm. Each hemolysis percentage was calculated with the equation (the absorbance – absorbance of the negative control)/(absorbance of the positive control – absorbance of the negative control) × 100.

Statistical Analysis

All data were presented as the mean ± SD and processed with ANOVA and Newman-Keuls by using Prism program.

Results and Discussion

Temperature Change Patterns in Different Kinds of Needles

Figure 2 shows that the temperatures of gold alloy needles rose faster and higher than those of SS304 needles. The highest temperature was 111°C recorded with Gold-1, and the lowest was 35.3°C with SS304-2. The temperature decrease after burning out the moxa also was sharper and greater in gold alloy needles than in SS304 needles. These results, however, seemed to be due to the difference in the conductivity of the materials and the diameters of the needles (SS304, 0.25 mm; gold alloy, 0.53 mm) and cannot be interpreted as a significant indication of any difference in the overall effect of warm-needling. Instead, the depth of insertion itself could be more significant since in the same needle the greatest temperature difference between two different heights was 33.5°C in SS304 needles and 42.2°C in gold alloy needles, even though the distance between the two points where the temperatures were measured was not >1 cm. This temperature difference reveals that in warm-needling clinical trials, the slightest difference in the height of the moxa chamber and the insertion depth would influence the intensity of heat delivered to the patient. Thus, considering the correlation between the heat and the effect of warm-needling, it is necessary to conduct

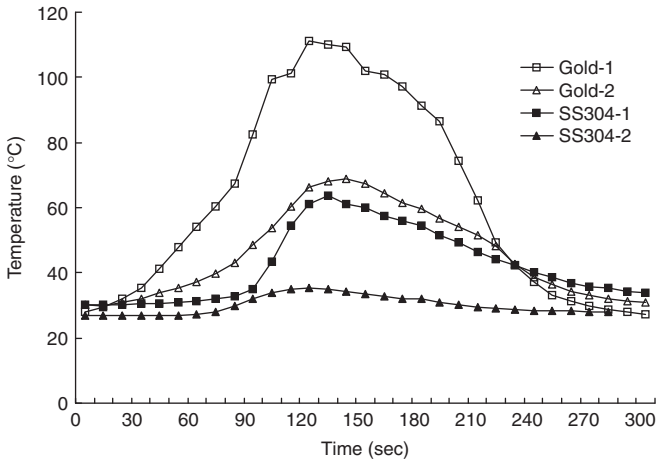


Figure 2. Temperature changes of thermo needles. Gold-1: 1 cm from the bottom of the handle part. Gold-2: 2 cm from the bottom of the handle part. SS304-1: 1 cm from the bottom of the handle part. SS304-2: 2 cm from the bottom of the handle part. The temperature of Gold-1 rose first, followed by Gold-2, SS304-1 and SS304-2 while the cooling curve displayed the characteristics of the material of acuneedle itself.

further research on the proper thickness and material of acuneedles for various applications of the technique.

Safety Test of SS304 Needles

Heavy metal, pH and cytotoxicity tests were performed to determine the safety of disposable SS304 acuneedles. To satisfy the KFDA standards for stents an ultraviolet absorbance spectrum measurement, tetrazolium-based colormetric test, hemolysis test and three kinds of biological tests were added.

To satisfy the KFDA safety standards, the difference between the pH of the extractant solutions and that of the standard solution should be within ± 1. All the pH measurements in this study fell within ± 1 of the standard solution's pH and satisfied the KFDA standards. Even though the pH differences of non-coated needles at all three temperatures were higher than those of the others, all of them were well within ± 1 (Table 1).

The heavy metal colormetric comparison test can detect Hg^{2+}, Pb^{2+}, Bi^{3+}, Cu^{2+}, Cd^{2+}, As^{3+}, Sb^{3+}, Sb^{5+}, Sn^{4+} and Sn^{2+}. In the color comparison, none of the extractant solutions showed a significant difference from the yellowish-brown transparent color of the standard solution and the colorless transparent blank test solution (Table 2). It was concluded, therefore, that all the extractant solutions were negative in terms of the existence of the above-mentioned heavy metal elements.

ICP–OES was carried out with silicone-coated needles, which are the most extensively used most regular acuneedles and warm needles. Two of the four tested elements, Fe and Sn, were < 5 p.p.m. each; Cu and Pb were not detected at all. Te had the greatest quantity among the four, but at most was only 0.1063 p.p.m., which is far less than the 0.5 p.p.m that KFDA sets as the standard (Table 3).

The ultraviolet absorbance spectrum measurement showed that the greatest absorbance of all the extractants was below 0.1 AU, satisfying the standard. The results, however, did not reveal any regular pattern, making it difficult to compare the absorbance measurements collected from different materials at different temperatures. For example, at 37°C, the absorbance order from highest to lowest was silicone-coated, salicine acid-coated and noncoated, but at 50°C, the order was salicine acid-coated, silicone-coated and noncoated. At 70°C, the noncoated and the salicine acid-coated had the same measurements and the silicone-coated was the lowest (Fig. 3).

Table 2. Colorimetric comparison results of the extractants of acuneedles

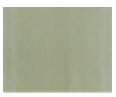
	Standard solution	Blank solution	
			
Material	Extraction temperature (°C)		
	37	50	70
Noncoated			
Silicone-coated			
Salicylic acid-coated			

Table 3. ICP–OES measurement results for extractant (p.p.m.)

Element	Wavelength (nm)	Extraction temperature (°C)		
		37	50	70
Cu	327.395	ND	ND	ND
Fe	238.204	0.0843 ± 0.0067	0.1063 ± 0.0027	0.0689 ± 0.0002
Pb	220.353	ND	ND	ND
Sn	283.998	0.0099 ± 0.0126	0.0252 ± 0.0268	ND

Wavelength, the wavelength at which each element was measured; ND, not detected. The data are presented as the mean ± SD.

Table 1. pH measurements of the extractants

Material	Range	MV	Extraction temperature (°C)								
			37			50			70		
			Range	MV	Diff.	Range	MV	Diff.	Range	MV	Diff.
Blank liquid	5.4–5.5	5.5									
Non-coated			5.8	5.8	0.3	5.8–5.9	5.9	0.4	6.1	6.1	0.6
Silicone-coated			5.5–5.7	5.6	0.1	5.6–5.7	5.7	0.2	5.8–5.9	5.9	0.4
Salicylic acid-coated			5.6–5.8	5.7	0.2	5.7–5.8	5.8	0.3	5.9–6.2	6.0	0.5

Range: The pH range of three measurements. MV, The midpoint value of 3 pH measurements; Diff., The difference between midpoint values of the measured sample and the blank sample. When the difference between the measured samples and the blank sample falls within ±1, it is considered to be compliant with 'The Standards for Disposable Needles'(KFDA). All samples measured here satisfied the requirement.

In the cytotoxicity test is there, the Cu extractant solution the positive control showed a greatest cytotoxity level than the negative control did. All the test results showed a significant difference from the positive control with a cytotoxicity of $P < 0.001$, but they did not show any significant difference from the negative control (SS304). Figure 4 shows that all the tested extractant solutions were negative.

The hemolysis test showed that the hemeolysis percentages of all the extractant solutions were negative, except for the salicine acid-coated extractant solution at 50°C. This result demonstrates that in all the extractant solutions except the salicine acid-coated extractant solution, the hemolysis occurred less than the positive control. Moreover, the only positive value exhibited by the salicine acid-coated solution at 50°C was 2.21%, which is far less than the 5%, standard maximum. Therefore, all the hemolysis percentages satisfied the KFDA standard (Table 4).

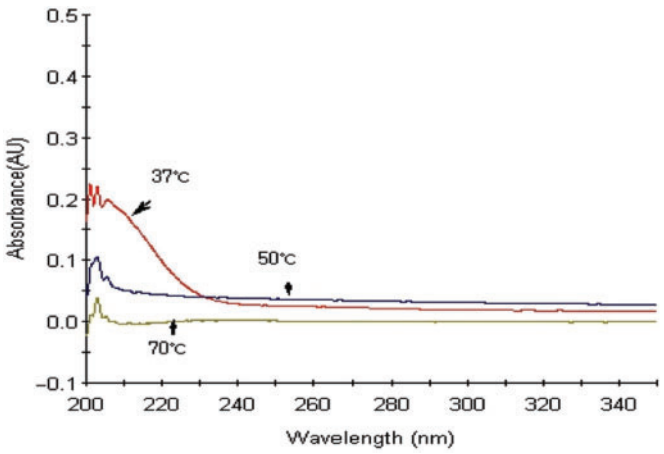


Figure 3. The absorbance versus wavelength curve for the extractants of the silicone-coated acuneedles. It should be noted that the extractant from each temperature fell below 0.1 AU between 250 and 350 nm.

Conclusion

The results of the tests conducted in this study indicate that all of the warm needles tested, regardless of their materials, are mechanically and biologically safe. Considering the rigid conditions this study imposed upon the process of preparing the extractant solutions, such solid results verifying the safety of warm needles are somewhat surprising. Given these results, it is expected that patients and practitioners will acquire more confidence in the warm-needling technique since the safety of the cheap disposable SS304 needles equals that of the traditional custom-made gold alloy needles.

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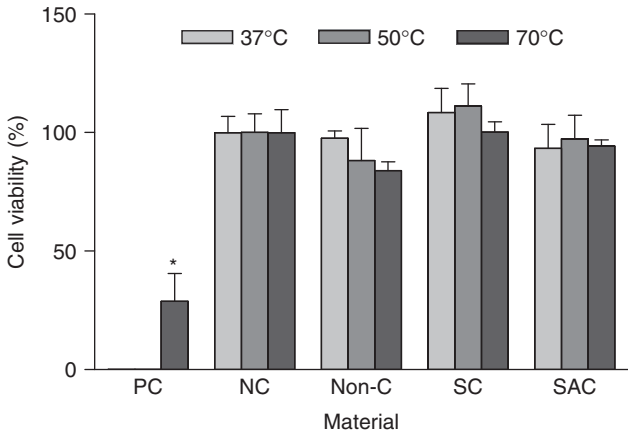

Figure 4. The viability of CCL81 (Vero) cells. PC: positive control, copper extractant. NC, negative control; SS304 extractant. Non-C; extractant from naked acuneedles. SC, extractant from silicon-coated acuneedles; SAC, extractant from salicylic acid-coated acuneedles. *$P < 0.001$ versus negative control.

Table 4. Hemolysis percentage calculated from absorbance

Material	Abs.	Hemolysis of needles					
		37°C		50°C		70°C	
		Abs.	Percentage of hemolysis	Abs.	Percentage of hemolysis	Abs.	Percentage of hemolysis
Positve control	0.46 ± 0.004						
Negative control	0.15 ± 0.004						
Non-coated		0.12 ± 0.003	−9.14	0.09 ± 0.001	−19.33	0.11 ± 0.002	−11.13
Silicone-coated		0.11 ± 0.002	−10.29	0.12 ± 0.003	−9.87	0.10 ± 0.002	−15.44
Salicylic acid-coated		0.10 ± 0.003	−15.65	0.15 ± 0.009	2.21	0.10 ± 0.001	−14.50

abs., absorbance at 540 nm. Values are expressed as the mean ± SD. Percentage of hemolysis = (absorbance of test sample-absorbance of negative control)/(absorbance of test sample − absorbance of positive control) × 100. If hemolysis percentage is under 5%, it is deemed to satisfy the stent requirements.

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